Supplementary Figure 1: STAT3 is required for rapid induction of *IL21* expression in naïve CD4$^+$ T cells by IL-12

(A-D) Sort purified naïve CD4$^+$ T cells from two normal donors and two STAT3$_{MUT}$ patients were cultured for 24 (A, C) and 48 (B, D) hours under neutral (nil) or Th1-polarising (Th1) conditions and expression of *IL21* (A, B) and *IFNG* (C, D) then determined by qPCR. Each symbol corresponds to data obtained from individual normal donors/patients.

Supplementary Figure 2: Mutations in *TYK2* impair IL-12 induced IL-21 expression

(A-E) The frequency of naïve (CD45RA$^+$CCR7$^+$), memory (CD45RA$^+$CCR7$^{-/+}$), and CXCR5$^+$CD45RA$^-$ CD4$^+$ T cells in PBMCs was determined for normal donors and TYK2-deficient patients. (A, B) representative dot plots from 1 donor and TYK2-deficient patient#1. (C-E) The frequency of (C) naïve (CD45RA$^+$CCR7$^+$), (D) memory (CD45RA$^+$CCR7$^{-/+}$), and (E) CXCR5$^+$CD45RA$^-$ CD4$^+$ T cells from all normal donors (total CD4$^+$ T cell, n=54; naïve CD4$^+$ T cell, n= 70; memory CD4$^+$ T cell, n=70; CXCR5 CD4$^+$ T cell, n=61) and TYK2-deficient patients (n=2).

(F-H) Total CD4$^+$ T cells from a normal donor and TYK2-deficient patient#1 were cultured for 5 days under neutral (nil) or Th1-polarising (IL-12) conditions and expression of intracellular IL-21 (F, G) and IFNγ (H) determined. The graphs in (F) and (H) show the frequency of cytokine-positive cells; those in (G) depict cytokine expression following Th1 polarisation as fold-increase relative to the nil culture in each experiment.

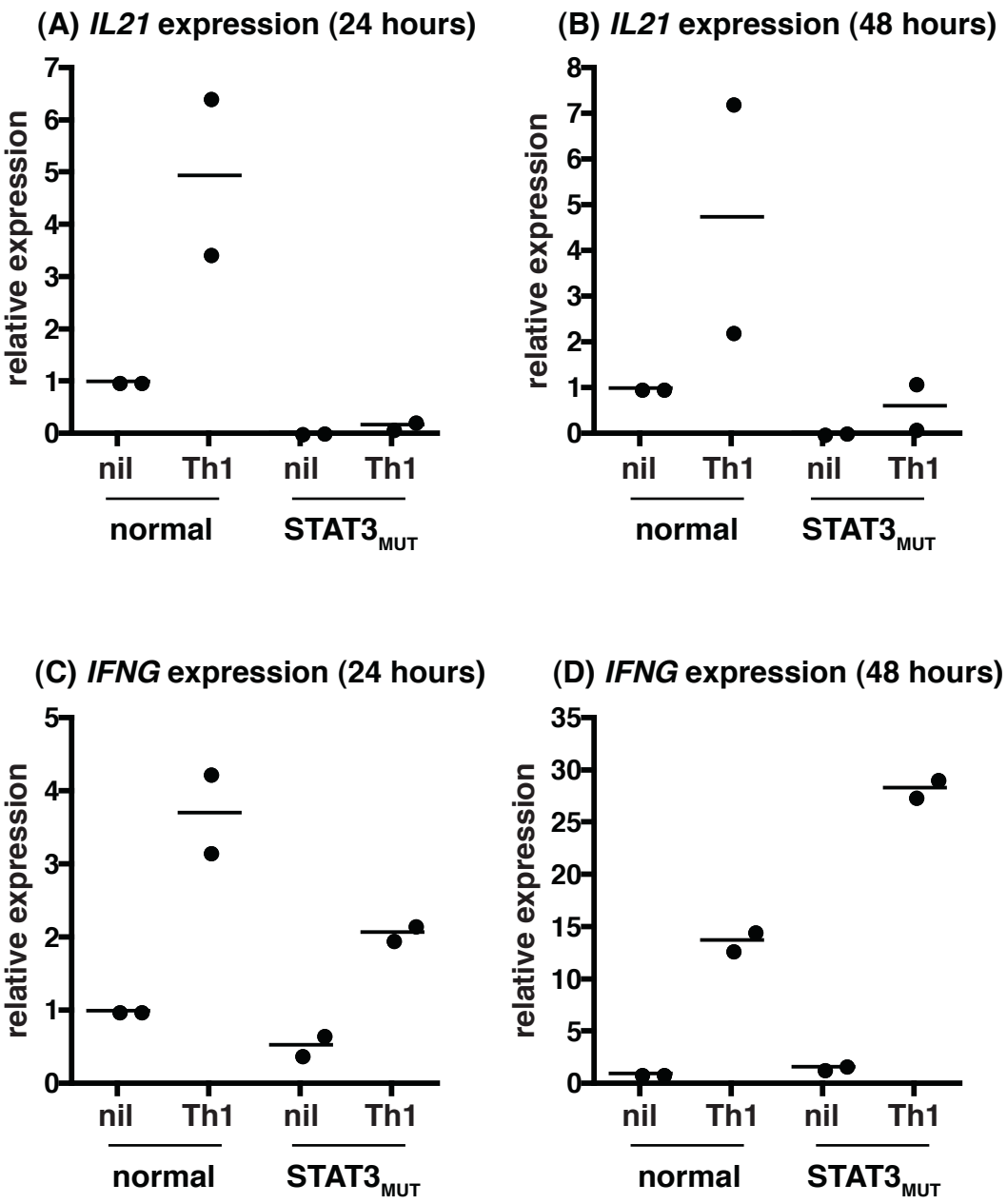
(I, J) Sort purified naïve CD4$^+$ T cells from a normal donor and TYK2-deficient patient#2 were cultured for 5 days under neutral (nil) or Th1-polarising (IL-12) conditions and expression of intracellular IL-21 (I) and IFNγ (J) determined.

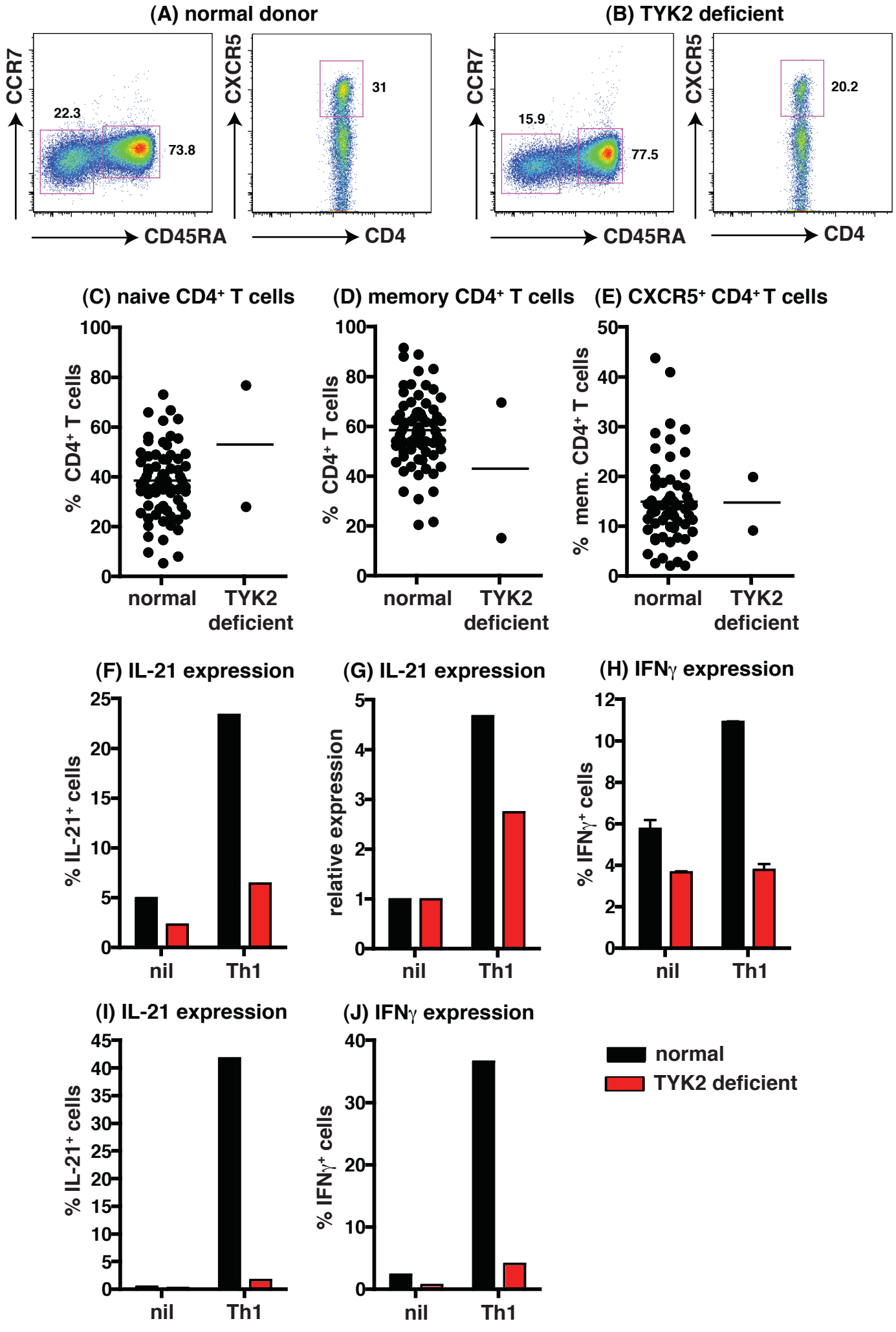
Supplementary Figure 3: *STAT3* mutations abolish Tfh function induced in vitro by IL-6, IL-21 and IL-23

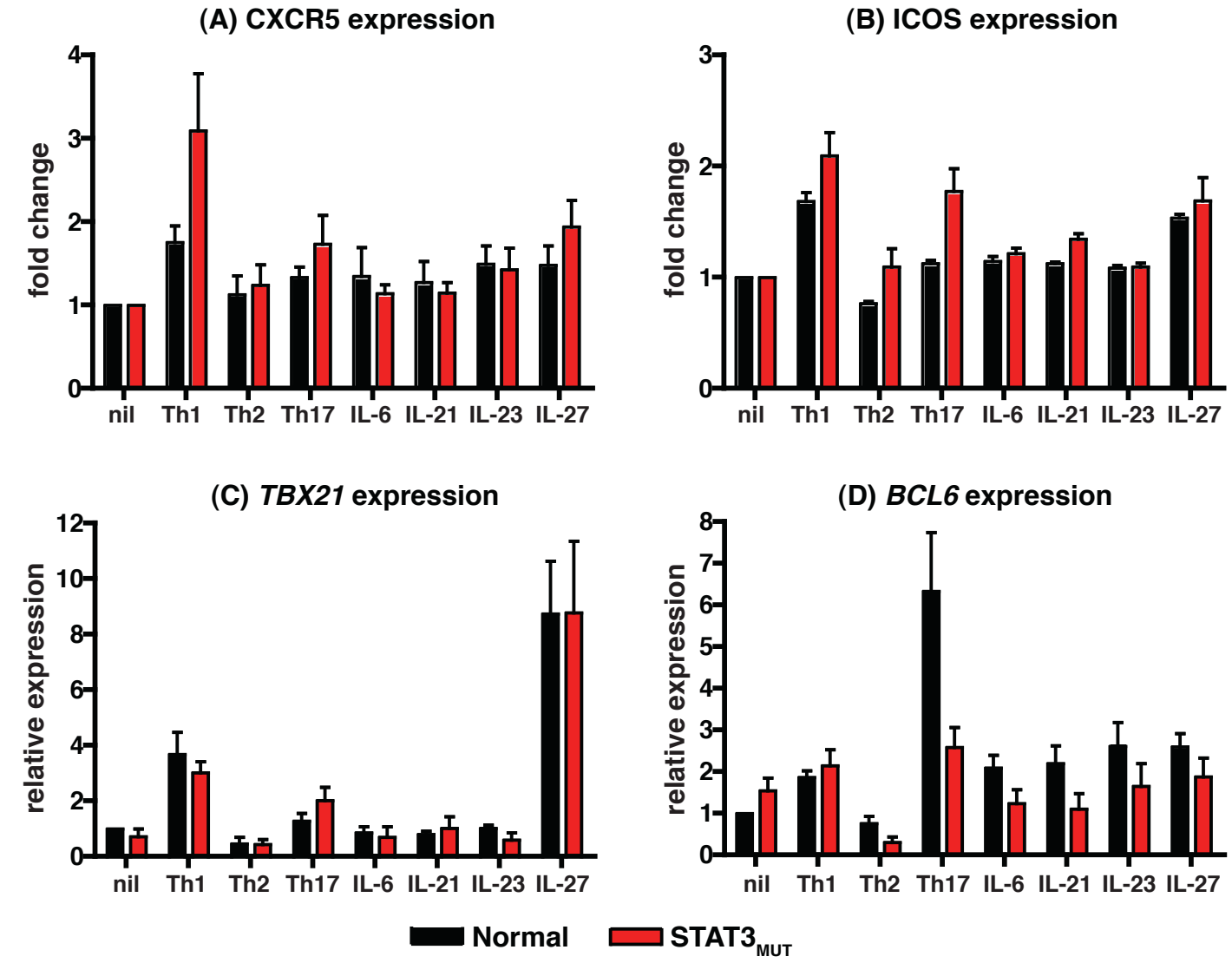
(A-D) Naive $CD4^+$ T cells isolated from normal donors and $STAT3_{MUT}$ patients were cultured under neutral conditions (nil), polarising Th1, Th2 or Th17 conditions or in the presence of IL-6, IL-21, IL-23 or IL-27. After 4 days, expression of (A) CXCR5, (B) ICOS (n=3), (C) *TBX21* (n=4) and (D) BCL6 (n=5) were determined by flow cytometry or qPCR.

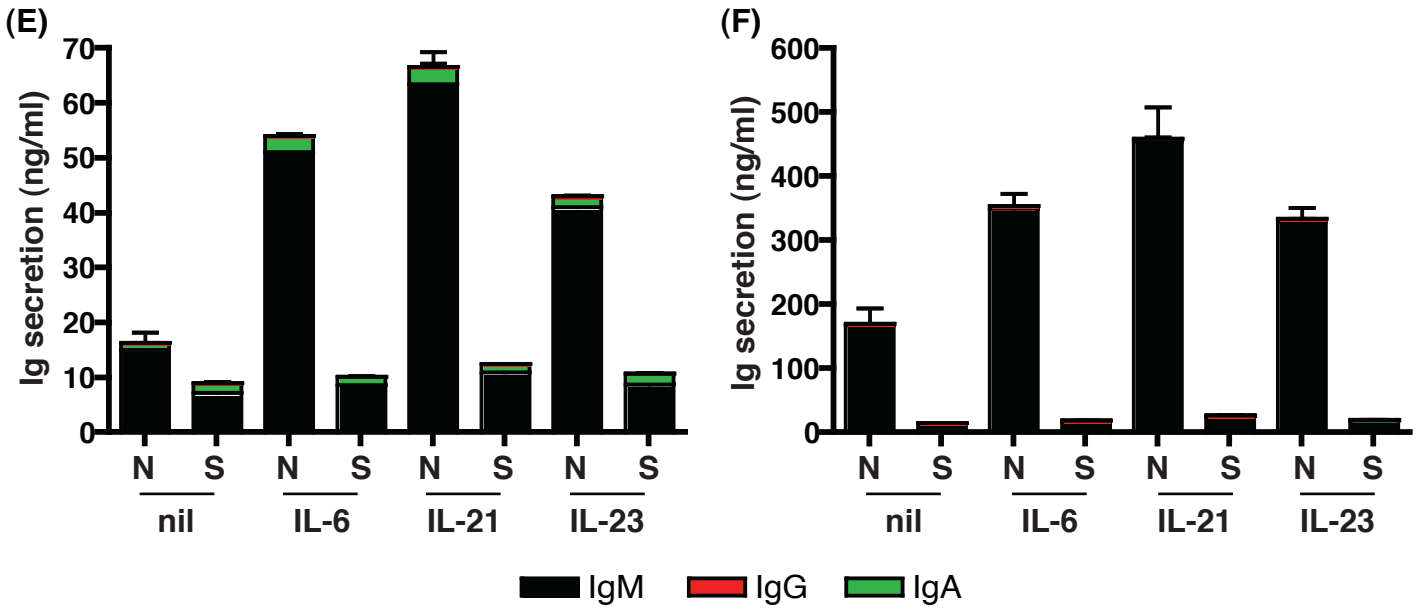
(E, F) Naive $CD4^+$ T cells isolated from normal donors (N) and $STAT3_{MUT}$ patients (S) were cultured under neutral (nil) conditions or with IL-6, IL-21 or IL-23 alone. After 5 days, the cells were harvested and treated with mitomycin C before being co-cultured with allogeneic naïve B cells for an additional 7 days. After this time secretion of IgM, IgG and IgA was determined. Error bars represent Ig values from triplicate wells; each graph is derived from $STAT3_{MUT}$ cells isolated from a different donor.

supplementary Figure 1




(A) normal donor
CCR7
22.3
73.8
CD45RA
CXCR5
31
CD4
(B) TYK2 deficient
CCR7
15.9
77.5
CD45RA
CXCR5
20.2
CD4
(C) naive CD4+ T cells
% CD4+ T cells
normal
TYK2 deficient
(D) memory CD4+ T cells
% CD4+ T cells
normal
TYK2 deficient
(E) CXCR5+ CD4+ T cells
% mem. CD4+ T cells
normal
TYK2 deficient
(F) IL-21 expression
% IL-21+ cells
nil
Th1
(G) IL-21 expression
relative expression
nil
Th1
(H) IFNγ expression
% IFNγ+ cells
nil
Th1
(I) IL-21 expression
% IL-21+ cells
nil
Th1
(J) IFNγ expression
% IFNγ+ cells
nil
Th1
normal
TYK2 deficient


(A) CXCR5 expression
fold change
0
1
2
3
4
nil
Th1
Th2
Th17
IL-6
IL-21
IL-23
IL-27
(B) ICOS expression
fold change
0
1
2
3
nil
Th1
Th2
Th17
IL-6
IL-21
IL-23
IL-27
(C) TBX21 expression
relative expression
0
2
4
6
8
10
12
nil
Th1
Th2
Th17
IL-6
IL-21
IL-23
IL-27
(D) BCL6 expression
relative expression
0
1
2
3
4
5
6
7
8
nil
Th1
Th2
Th17
IL-6
IL-21
IL-23
IL-27
Normal
STAT3MUT


(E)
Ig secretion (ng/ml)
0
10
20
30
40
50
60
70
N
S
nil
IL-6
IL-21
IL-23
(F)
Ig secretion (ng/ml)
0
100
200
300
400
500
600
N
S
nil
IL-6
IL-21
IL-23
IgM
IgG
IgA